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Effects of Parathyroid Hormone on H^+ and NH_4^+ Excretion in Toad Urinary Bladder

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Summary. The urinary bladder of *Bufo marinus* excretes H^+ and NH_4^+, and the H^+ excretion is increased after the animal is placed in metabolic acidosis. The present study was done to determine if parathyroid hormone could stimulate the bladder to increase the excretion of H^+ and/or NH_4^+. Parathyroid hormone added to the serosal solution in a final concentration of 10 μg/ml was found to increase H^+ excretion by 50% above the control hemibladders, while there was no effect on NH_4^+ excretion. Parathyroid hormone had no effect on H^+ excretion when added to the mucosal solution. We also performed experiments utilizing theophylline and dibutyryl cyclic AMP which mimicked those of the parathyroid hormone experiments. A dose-response analysis was performed and the results indicate that 1 μg/ml of parathyroid hormone was the minimal effective dose. These results suggest that parathyroid hormone can stimulate H^+ excretion in the toad urinary bladder and this effect seems to be mediated by cyclic AMP. In addition, it was found that parathyroid hormone has no effect on NH_4^+ excretion.

It has been shown previously that the toad urinary bladder is capable of excreting H^+ (Frazier & Vanatta, 1971; Ludens & Fanestil, 1972). In a later study, Frazier and Vanatta (1972) demonstrated that it was possible to stimulate H^+ excretion and NH_4^+ excretion in the toad urinary bladder by using plasma and extracts of plasma from toads in metabolic acidosis. The effects of parathyroid hormone (PTH) on calcium and phosphorus in the mammalian nephron are well known (Rasmussen & Bordier, 1974). In addition, there have been several reports in the literature that PTH is important in controlling the rate of bicarbonate reabsorption in the mammalian nephron (Hellman, Au & Bartter, 1965; Wachman & Berstein, 1970; Barzel, 1971; Diaz-Buxo & Knox, 1975).

The purpose of the present study was to determine if PTH has any effect on H^+ and NH_4^+ excretion in the toad urinary bladder. Using paired hemibladders we found that PTH stimulated H^+ excretion significantly without a similar effect on NH_4^+ excretion. Experiments performed with the phosphodiesterase inhibitor, theophylline and experiments with

dibutyryl cyclic AMP, indicate that PTH effect is mediated by cyclic AMP. In additional experiments we found that the PTH could exert this effect only when present on the serosal surface of the bladder.

Materials and Methods

The toads used in these experiments were *Bufo marinus* of Colombian origin and were supplied by Charles P. Chase of Miami, Florida. The routine care of toads, solutions, the procedure of inducing acidosis, and the method of measuring H^+ and NH_4^+ excretion were as previously described (Frazier & Vanatta, 1973). In all experiments, the H^+ excretion was calculated from change in pH and the concentration of buffer in the mucosal solution. The H^+ excretion was calculated using a pKa for the phosphate buffer pair of 6.50. This value was obtained both empirically in Ringer's solution and by calculation to correct for the ionic strength (Frazier & Vanatta, 1973). A Radiometer Model PHM 64 digital pH meter was used for all pH determinations. One hundred per cent humdified O_2 was bubbled into the mucosal medium throughout each experiment. The Ringer's solution used contained in mM: NaCl, 114.5; KCl, 3.0; $CaCl_2$, 0.9; and sodium phosphate, 1.5; the final pH was 6.80. The parathyroid hormone was obtained from Sigma Chemical Co., St. Louis, Missouri, as the trichloroacetic acid (TCA) powder and contained an activity of 263 USP parathyroid units per milligram. The dibutyryl cyclic AMP and theophylline were also obtained from Sigma Chemical Co. All statistics were performed as the mean difference on paired hemibladders using Student's *t* test.

Paired hemibladders from toads in normal acid-base balance were used as the assay system except in one case where bladders from toads in metabolic acidosis were used. The hemibladders were mounted between lucite chambers, each of which held 2 mm. The cross sectional area of each chamber was 1.98 cm^2. The serosal bath of the experimental hemibladder contained a phosphate buffered Ringer's solution to which had been added the indicated amount of PTH, theophylline or dibutyryl cyclic AMP. The control hemibladder contained only the phosphate buffered Ringer's solution in the serosal bath.

The mucosal chamber contained a 1.5 mM phosphate buffered Ringer's solution. The flux period was for 120 min. Ammonium and pH were determined on each mucosal and serosal sample both before and after the flux period. The flux was started after a 30 min equilibration period, during which time the bladder was exposed to the PTH, theophylline or dibutyryl cyclic AMP.

Results

Effect of PTH on H^+ and NH_4^+ Excretion

In the first experiments PTH (10 μg/ml) was placed in the serosal medium. In Table 1 are shown the results of this experiment. As can be seen, PTH in the serosal medium increased H^+ excretion by approximately 50% over the control value ($p < 0.025$). In contrast to this, PTH had no significant effect on the excretion of NH_4^+ by the bladder ($p > 0.50$).

Table 1. Effect of PTH in serosal media on H^+ and NH_4^+ excretion in the toad urinary bladder (excretion in nmoles/100 mg bladder × min)

Serosal solution	H^+ excretion[a]	Mean difference[b] with (p value)[c]	NH_4^+ excretion	Mean difference with (p value)
Experiments using bladders from normal toads				
Ringer's solution	8.38		1.04	
		4.33 ± 1.61 ($p < 0.025$)		0.03 ± 0.19 ($p > 0.50$)
Ringer's solution with PTH (10 μg/ml)	12.71		1.02	
Experiments using bladders from toads in metabolic acidosis				
Ringer's solution	13.81		2.99	
		2.96 ± 2.35 ($p > 0.10$)		0.83 ± 0.79 ($p > 0.05$)
Ringer's solution with PTH (10 μg/ml)	16.77		2.16	
Experiments using bladders from normal toads with heat inactivated PTH in the serosal media				
Ringer's solution	8.33		1.42	
		-0.48 ± 2.42 ($p > 0.20$)		0.44 ± 0.36 ($p > 0.20$)
Ringer's solution with heat inactivated PTH (10 μg/ml)	7.87		1.86	

[a] Average of ten experiments.
[b] ±SEM.
[c] Calculated from the mean difference.
[d] PTH inactivated at 56 °C for 15 min.

The serosal base excretion in this series of experiments, as calculated from change in pH of the serosal medium, averaged 4.21 ± 0.92 nmoles/100 mg bladder × min. The serosal base excretion was not affected by PTH, the mean difference between control and experimental bladders was 0.18 ± 2.34 ($p > 0.50$). Likewise, in the remainder of the studies, serosal base excretion was not affected by the experimental intervention.

Also shown in Table 1 is a second group of experiments performed in an identical manner as the experiment given above. However, the bladders used in this experiment were from toads which had been placed in metabolic acidosis 48 hr earlier by NH_4Cl loading. As can be seen in Table 1 there was no apparent stimulation of H^+ excretion by the PTH in the bladders from toads in metabolic acidosis ($p > 0.10$). Likewise, the NH_4^+ excretion was not stimulated in this series of bladders ($p > 0.05$).

Table 2. Effect of PTH in mucosal media on H^+ and NH_4^+ excretion in the toad urinary bladder (excretion in nmoles/100 mg bladder × min)

Mucosal solution	H^+ excretion[a]	Mean difference[b] with (p value)[c]	NH_4^+ excretion	Mean difference with (p value)
Ringer's solution	17.12		0.35	
		0.12 ± 3.23 ($p > 0.40$)		0.08 ± 0.13 ($p > 0.40$)
Ringer's solution with PTH (10 μg/ml)	17.24		0.28	

[a] Average of nine experiments.
[b] ±SEM.
[c] Calculated from the mean difference.

Table 1 also contains a group of experiments performed in an identical manner to those above except that the PTH had been heat inactivated before it was placed on the bladder. The PTH was inactivated by placing it in a 56 °C water bath for 15 min. It is apparent from the results that the heat inactivated PTH had no effect on either H^+ or NH_4^+ excretion ($p > 0.20$; $p > 0.20$, respectively).

A third group of experiments was performed to determine if PTH would also stimulate H^+ excretion when present on the mucosal surface of the bladder and not present on the serosal surface. Table 2 shows the results of this experiment. These experiments were done in a manner identical to those presented previously except that the PTH was placed in the mucosal medium. As can be seen, the PTH in the mucosal medium had no significant effect on either H^+ or NH_4^+ excretion ($p > 0.40$).

The Effect of Theophylline and Dibutyryl Cyclic AMP on H^+ and NH_4^+ Excretion

It has been reported by Rasmussen and Bordier (1974) that the effect of PTH on bone and renal tubules is mediated by cyclic AMP. In addition, PTH stimulates adenyl cyclase and causes an increase in the concentration of cyclic AMP within renal tubular cells (Shlatz, Schwartz, Kinne-Saffran, Kinne, 1975). In order to determine if cyclic AMP is involved in the PTH response of the bladder, a series of experiments was performed in which theophylline, an inhibitor of phosphodiesterase, was added to the serosal medium. The results are shown in Table 3. It is apparent that theophylline stimulates H^+ excretion, and the response is very similar

Table 3. Effect of theophylline and dibutyryl cyclic AMP (dBC-AMP) on H^+ and NH_4^+ Excretion in toad urinary bladder (excretion in nmoles/100 mg bladder × min)

Serosal solution	H^+ excretion[a]	Mean difference[b] with (p value)[c]	NH_4^+ excretion	Mean difference with (p value)
		Experiments Using Theophylline		
Ringer's solution	7.39		1.08	
		4.18 ± 0.84 ($p < 0.05$)		0.42 ± 0.28 ($p > 0.05$)
Ringer's solution with Theophylline (10^{-3} M)	11.57		1.50	
		Experiments Using Theophylline and PTH		
Ringer's solution	13.76		0.92	
		8.70 ± 1.37 ($p < 0.001$)		0.14 ± 0.16 ($p > 0.20$)
Ringer's solution$^+$ PTH (10 μg/ml$^+$ Theophylline (10^{-3} M)	22.45		1.05	
		Experiments Using dBC-AMP		
Ringer's solution	16.38[d]		1.53	
		11.8 ± 2.17 ($p < 0.001$)		0.48 ± 0.27 ($p > 0.05$)
Ringer's solution with dBC-AMP (10^{-3} M)	28.19[d]		1.05	

[a] Average of ten experiments.
[b] ±SEM.
[c] Calculated from the mean difference.
[d] Average of eight experiments.

to that obtained when using PTH ($p < 0.05$). Again, there was no significant stimulation of NH_4^+ excretion in this experiment ($p > 0.05$). These results suggest that the action of PTH might indeed be mediated by cyclic AMP.

If this is true, then one might assume that theophylline and PTH together could augment the excretion of H^+. Table 3 shows the results of experiments performed using both PTH and theophylline in the serosal medium. This intervention resulted in approximately a 63% increase in H^+ excretion over the control ($p < 0.001$). The NH_4^+ excretion was not effected in this series of experiments ($p > 0.20$).

Also shown in Table 3 are the results obtained when dibutyryl cyclic AMP was placed in the serosal media. It is obvious that dibutyryl cyclic

Table 4. Dose-response analysis of the effect of PTH on H^+ excretion

PTH Concentration μg/ml (serosal solution)	ΔH^+ Excretion[a] (PTH-control), nmoles/100 mg bladder × min	*P* value
0.1	2.15 ± 1.38 (n = 8)	> 0.05
1.0	2.29 ± 1.03 (n = 8)	< 0.05
10.0	4.33 ± 1.62 (n = 10)	< 0.025
100.0	7.28 ± 1.63 (n = 8)	< 0.002

[a] Values are the mean difference between paired hemibladders one receiving PTH and the other no PTH (control) ± SEM.

AMP significantly increases H^+ excretion in the toad bladder ($p < 0.001$). Again, there was no apparent effect on NH_4^+ excretion ($p > 0.05$).

Dose-Response Analysis of PTH

A dose-response analysis was performed and the results are shown in Table 4. The concentration of PTH was varied from 0.1 to 100 μg/ml in the serosal media. ΔH^+ excretion values represent the mean difference between paired hemibladders, one receiving PTH and the paired hemibladder receiving no PTH. The *p* values represent the probability that the ΔH^+ excretion is different from zero. The results show that (1) at 0.1 μg/ml a positive but insignificant ΔH^+ was observed ($0.10 > p > 0.05$); (2) at 1 μg/ml PTH increased H^+ excretion in the border line level of significance ($p < 0.05$); (3) at 10 μg/ml dose of PTH there was further stimulation of H^+ excretion ($p < 0.025$); (4) at 100 μg/ml there was also further stimulation ($p < 0.002$).

Discussion

There have been several reports in the literature that indicate that PTH is important in acid-base balance in the mammal (Hellman *et al.*, 1965; Wachman & Bernstein, 1970; Barzel, 1971; Diaz-Buxo & Knox, 1975). The report by Wachman and Bernstein (1970) indicates that PTH plays a role in acid-base metabolism through its regulation of phosphate

metabolism, e.g., increasing the renal clearance of phosphate. However, all of the other studies mentioned above show that PTH has a direct effect of reducing HCO_3^- reabsorption in the mammalian nephron, since HCO_3^- reabsorption is the result of H^+ excretion, PTH must inhibit H^+ excretion in the mammalian nephron. It has been shown that the toad urinary bladder is capable of excreting H^+ and NH_4^+ (Frazier & Vanatta, 1971). Since this is an analogous function to the mammalian nephron, these studies were done to determine if PTH could have an effect on H^+ and/or NH_4^+ excretion.

Our studies show that PTH does stimulate H^+ excretion in the toad urinary bladder. This is opposite the response observed in the mammalian nephron, where HCO_3^- reabsorption is inhibited by PTH. The reason for this difference is not clear at the present time. It should be pointed out that this effect of PTH in the mammalian nephron is thought to be localized in the proximal tubule (Karlinsky, Sager, Kurtzman and Pillay, 1974; Diaz-Buxo & Knox, 1975). The toad urinary bladder is generally considered to be analogous in function to the distal nephron. Therefore, it is not surprising that the response to PTH in the toad is different from that reported for the mammal.

In Table 1 it was noted that PTH stimulated H^+ excretion in the bladders from toads in normal acid-base balance and not in bladders from toads in chronic metabolic acidosis. The reason PTH failed to increase H^+ excretion in the bladders from toads in metabolic acidosis is not apparent at this time. It is possible that the H^+ secretory mechanisms were already stimulated to maximum rates by the metabolic acidosis. It might also be argued that the acidic environment of the tissue might have in some way prevented the action of PTH on the bladder.

Our results show that PTH stimulates H^+ excretion when it is present on the serosal surface of the bladder. It is concluded that the receptors for PTH are accessible only from the serosal side of the bladder. Whether the receptors are on the serosal plasma membrane or inside the cell cannot be concluded from these experiments. It is interesting, however, to note a recent report by Shlatz *et al.* (1975) which reports that adenylate cyclase, specifically stimulated by PTH, is distributed in the contraluminal region of the membrane of mammalian renal cortical epithelial cells.

The results of these studies suggest that the response of the toad urinary bladder to PTH may be mediated by cyclic AMP. Theophylline, a phosphodiesterase inhibitor, stimulated H^+ excretion to about the same degree as PTH. In addition, the effect of PTH could be augmented by combining it with the theophylline. Dibutyryl cyclic AMP was also

effective in eliciting this increased H^+ excretion in the toad bladder.

Caution must be exercised when interpreting this data concerning cyclic AMP. It is possible that PTH could be stimulating another metabolic pathway in the bladder, thus increasing CO_2 production in the bladder. It has been reported (Frazier, 1974) that H^+ excretion is dependent on the metabolic CO_2 production of the bladder. However, it was shown in this same report that vasopressin, whose action is mediated by cyclic AMP, has no effect on H^+ excretion in toad bladder. It seems then that the PTH response is mediated specifically by cyclic AMP in the toad urinary bladder and not indirectly by stimulating an alternate metabolic pathway.

There was a wide variability observed between experimental groups with regards to H^+ excretion. In Table 1 with bladders from toads in metabolic acidosis the rate of H^+ excretion was 16.77, whereas in Table 3 using theophylline and dBC-AMP considerably higher rates were observed. Various factors could be contributing to this variation. It is well known that animals collected in the field show more variation than inbred laboratory strains. In addition, amphibia are known to show marked seasonal variation. Since we used paired hemibladders and looked only at the difference between the paired halves of each bladder, this variability observed between groups should not affect our conclusions.

The stimulatory effect of PTH on H^+ excretion could be nonspecific and be elicited by any peptide compound. However, this seems highly unlikely for two reasons; (1) the results in Table 1, using heat inactivated PTH, were clearly negative; and (2) previous experiments (Frazier, 1974) have shown that vasopressin has no effect on H^+ excretion. It seems then that the stimulatory effect of PTH on the H^+ excretory system is a specific one.

The evidence presented here is consistent with the proposal that PTH increased the capacity of the toad urinary bladder to excrete H^+. Whether this effect is relevant in the overall acid-base physiology of the toad requires (1) that the toads have parathyroid hormone and that it be increased in response to metabolic acidosis and (2) that the dose-response analysis for the *in vitro* effect of PTH on H^+ excretion be within the physiological range. Moore (1964) reports that adult amphibians have parathyroid tissue and further, this is the first vertebrate group phylogenetically, in which such glands are found as organized bodies. We were unable to find any reports in the literature concerning PTH levels in the toads during metabolic acidosis. It is interesting to note that in humans it has been reported that PTH is increased in response to a

metabolic acidosis (Wachman & Bernstein, 1970). The dose-response analysis (Table 4) showed that 0.1 μg/ml PTH had no effect on H^+ excretion, that 1 μg/ml produced a small but significant effect. We were unable to find any reported levels of PTH in the amphibians. The PTH concentrations used in our experiments are very similar to those used by Borle (1970) and Nagata and Rasmussen (1970) to stimulate calcium flux and gluconeogenesis in mammalian renal tubules *in vitro*. The dose-response relationship for the effect of PTH on H^+ excretion appeared to be applicable in the sense that it was within the range accepted for mammalian systems. Obviously additional tests with more stringent criteria are needed to absolutely implicate the direct effect of PTH in acid-base balance of the toad.

It should be noted in the first experiments with PTH on the serosal surface, that serosal alkalization did not increase with PTH stimulation of mucosal acidification. It could be that the PTH itself acted as a buffer in the serosal medium since it was only present in the experimental serosal medium and not the control serosal medium. It is also possible that the PTH in some way changed the intracellular buffering capacity of the bladder mucosal cell. It is not apparent from these studies why serosal alkalization failed to increase concurrently with mucosal acidification.

In summary, our studies have shown (1) that PTH will stimulate H^+ excretion in the toad urinary bladder, without a similar effect on NH_4^+ excretion; (2) bladders from toads in chronic metabolic acidosis appear to be insensitive to further stimulation by PTH; (3) the effect of PTH on the bladder may be mediated by cyclic AMP; (4) PTH has the ability to stimulate H^+ excretion in the bladder only when present on the serosal surface.

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